



# Effect of Consciousness Energy Healing Treated (The Trivedi Effect®) Novel Formulation on Bone Health: Role of Alkaline Phosphatase (ALP)

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## Abstract

Bone health is one of the important parts of healthy-life and longevity. In the current scenario, due to indiscriminate uses of pesticides in agriculture, there is a high abundance of toxins and contaminants in the environment and food chain. For this consequence, the present study has investigated the effect of the Biofield Energy Healing (The Trivedi Effect®) Based test formulation on bone health parameters using the human bone osteosarcoma cells - MG-63(ATCC® CRL-1427™). A proprietary formulation was designed that consisted of eight ingredients viz. zinc chloride, ferrous sulfate, sodium selenate, nanocurcumin, copper chloride, magnesium gluconate, vitamin C (ascorbic acid), and vitamin D<sub>3</sub> (cholecalciferol). The test formulation was divided into two parts. One part was denoted as the untreated test formulation without any Biofield Energy Treatment, while the other part was defined as the Biofield Energy Treated sample, which received the Biofield Energy Healing Treatment by renowned Biofield Energy Healer, Mahendra Kumar Trivedi. The cell viability assay data of the test formulation showed more than 70% cell viability at the concentration ranges from 0.001 to 10 µg/mL, which indicated its safe and non-cytotoxic nature. Alkaline phosphatase enzyme (ALP) was significantly increased by 80.16%, 292.91%, 200.36%, 22.24%, and 690.35% in the Biofield Energy Treated test formulation group (G4) at 0.0008, 0.001, 0.0033, 0.052, and 10.41 µg/mL, respectively as compared to the untreated test formulation group (G3). As a result of that phosphorus absorption and deposition in bone cells can be increased which can help to make stronger bone. The data demonstrated that the Biofield Treatment has the strong potential for the treatment of patients with arthritis, osteoporosis, and other bone disorders. Thus, Consciousness Energy Healing can be useful as a bone cells growth promoter for different bone-related disorders like low bone density, osteogenesis imperfecta, osteoporosis, etc.

**Keywords:** The Trivedi Effect®; Biofield Energy Healing; Osteosarcoma cells - MG-63; Alkaline phosphatase; MTT; ELISA

## Abbreviations

PTH: Parathyroid hormone; PGE2: Prostaglandin E2; ALP: Alkaline phosphatase; BAP: Bone alkaline phosphatase; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS: Phosphate buffer saline; ELISA: Enzyme-linked immunosorbent assay; CD-FBS: Charcoal-dextran treated fetal bovine serum

## Introduction

The bone remodeling is a continuous process, in which bone degradation or resorption is balanced by bone formation. This process is required for maintaining bone health. If the process is disturbed and the rate of bone resorption exceeds than the rate of bone formation, that cause's bone loss and lead to osteoporosis [1,2]. Osteoporosis is a metabolic bone disease characterized by

low bone mass and abnormal bone micro architecture. These two abnormal conditions occurs due to various number of clinical conditions viz. states of high bone turnover, hyperparathyroidism, thyrotoxicosis, osteomalacia, renal failure, gastrointestinal diseases, multiple myeloma, etc. [3,4]. The human osteosarcoma cells (MG-63) is used extensively to study the production of the osteocalcin, a bone-specific protein [5]. Literature suggests that parathyroid hormone (PTH) and prostaglandin E2 (PGE2) can inhibits osteocalcin secretion through cAMP production. Both the cell cultures showed time and dose-dependent stimulation of osteocalcin secretion in response to 1,25-dihydroxyvitamin D3 (1,25(OH)<sub>2</sub>D<sub>3</sub>) active form of vitamin D<sub>3</sub>. Thus, MG-63 cells are extensively used as an alternative to osteoblast-like cell model to study the regulation of osteocalcin secretion [6,7]. Alkaline phosphatase (ALP) has different functions in various organisms and tissues and plays a significant role in the initial phases of the bone mineralization process. Bone alkaline phosphatase (BAP) is the bone-specific isoform of ALP. A glycoprotein that is found on the surface of osteoblasts. BAP reflects the biosynthetic activity of these bone-forming cells. ALP plays a crucial role in the process of mineralization. The mechanisms through which ALP expression regulated are complex through WNT signaling cascade, chondrogenesis, and BMP/RUNX2 (CBAf1, AML3)/Osterix system [8,9]. Although different treatment strategies have been available, however an alternative "Energy Therapy" is one of the best approach for the management of osteoporosis in a cost-effective manner compared to the other conventional therapy. In the current world scenario, Energy Therapy like "Biofield Energy Healing" has been widely used and recommended as an

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alternative method that has an impact on various properties of living organisms in a very cost-effective manner [10]. The Trivedi Effect<sup>®</sup> has improved the overall productivity of crops in agriculture and livestock [11-14], positive impact on cancer [15,16], and altered characteristics features of microbes in the field of microbiology [17-20]. It also changed the structural, physical, and thermal properties of several metals and ceramics [21-23], causes a genetic alteration in microbes [24,25], and improves various nutraceutical compounds in the areas of nutraceuticals [26,27] and biotechnology [28-30]. Numerous treatment approaches are present nowadays for the growth of bone cells using synthetic drugs and chemicals. Alternative therapy like Biofield Energy Therapy is one of them. In this context, authors planned to investigate the effect of Biofield Energy Healing (The Trivedi Effect<sup>®</sup>) treated test formulation on bone health using human bone osteosarcoma cells - MG-63 (ATCC<sup>®</sup> CRL-1427<sup>™</sup>).

## Materials and Methods

### Chemicals and Reagents

Antibiotics solution (penicillin-streptomycin) and DMEM (phenol red-free) were procured from HiMedia. DMEM was procured from GIBCO, USA. Direct Red 80, 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium (MTT), and  $\beta$ -Estradiol (positive control) were purchased from Sigma Chemical Co. St. Louis, MO. Iron sulfate, copper chloride, cholecalciferol, streptozotocin, cyclophosphamide, and sodium carboxymethyl cellulose were obtained from Sigma Chemical Co. (St. Louis, MO). Nanocurcumin was purchased from Sanat Products Ltd., India. Zinc chloride and magnesium (II) gluconate hydrate were obtained from TCI, Japan. Sodium selenate and ascorbic acid were procured from Alfa Aesar, USA. All the other chemicals used in this experiment were analytical grade procured from India.

### Maintenance of Culture

Human bone osteosarcoma cell line -MG-63 (ATCC<sup>®</sup> CRL-

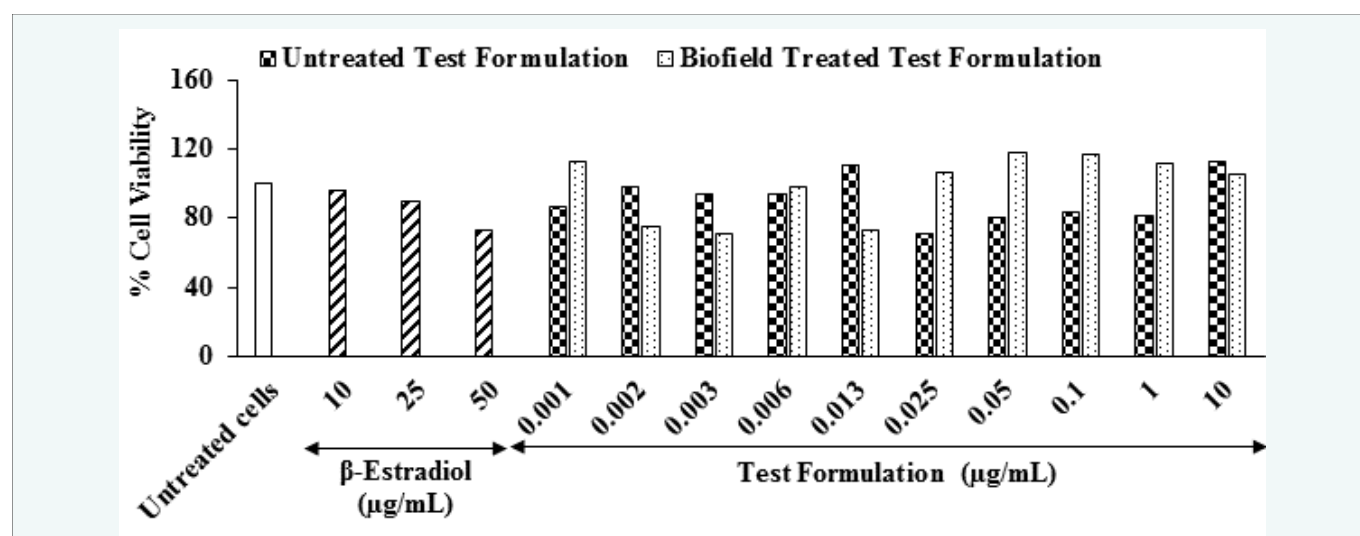
1427<sup>™</sup>) was used as a test system. The cells were maintained using DMEM growth medium with 10% FBS. The culture conditions were regulated at 37°C, 5% CO<sub>2</sub>, and 95% humidity and subcultured by trypsinization followed by splitting the cell suspension into a new flasks and supplementing with new cell growth medium. Three days before the start of the experiment (i.e., day -3), the growth medium of near-confluent cells was replaced with fresh phenol-free DMEM, supplemented with 10% charcoal-dextran stripped FBS (CD-FBS) and 1% penicillin-streptomycin [31].

### Biofield Energy Healing Approach

The test formulation was a combination of eight ingredients viz. zinc chloride, ferrous sulfate, sodium selenate, nanocurcumin, copper chloride, magnesium gluconate, vitamin C (ascorbic acid) and vitamin D<sub>3</sub> (cholecalciferol). Each ingredient of the test formulation was divided into two parts. One part of each ingredient was considered as untreated test formulation, where no Biofield Energy Healing Treatment was provided to these ingredients. Further, the untreated group was treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Healing Treatment. The second part of each ingredient received Biofield Energy Healing Treatment (known as The Trivedi Effect<sup>®</sup>) under laboratory conditions for 3 minutes through Mahendra Kumar Trivedi's unique Energy Transmission process to the test formulation. Biofield Energy Healer in this study did not visit the laboratory, nor had any contact with the herbomineral samples. After that, the Biofield Energy Treated and untreated ingredients were kept in similar sealed conditions and used for the study as per the study plan.

### Experimental Design

The tested cells were divided into four groups. Group 1 was served as baseline control (untreated cells with 200  $\mu$ L of



**Figure 1** Effect of the novel test formulation on human bone osteosarcoma cells (MG-63) for the evaluation of its proliferation using MTT cell viability assay. G1: Untreated cells; G2: Positive control ( $\beta$ -Estradiol); G3: Untreated test formulation. G4: Biofield Energy Treated test formulation.



phenol-free DMEM supplemented with 10% CD-FBS). Group 2 was served as positive control ( $\beta$ -estradiol). Group 3 and 4 were defined as the untreated and Biofield Energy Treated test formulation groups, respectively in phenol-free DMEM medium supplemented with 10% CD-FBS.

### Assessment of Cell Proliferation

The MG-63 cells were trypsinized, counted, and plated in 96-well plates (at  $5 \times 10^3$  cells/well/180  $\mu$ L) of growth medium as per Trivedi et al. 2018 [32]. The absorbance was recorded at 540 nm using microplate reader by Synergy HT [33]. The cell proliferation was determined by this equation (1):

$$\% \text{ Cell growth} = \left[ \frac{[(X-Tz)/(R-Tz)] \times 100}{1} \right] \quad (1)$$

Where, X = Absorbance of cells corresponding to positive control and test groups after 48 hours

R = Absorbance of baseline cells group (at 48 hours)

Tz = Absorbance of untreated cells (at 0 hour)

The positive control was performed simultaneously and the experiment was repeated thrice.

### Assessment of Alkaline Phosphatase (ALP) Activity

The MG-63 cells were trypsinized, counted, and plated in 96-well plates (at  $10 \times 10^3$  cells/well/180  $\mu$ L) of growth medium as per Trivedi et al. 2018 [32]. The absorbance was recorded at 405 nm by Synergy HT microplate reader (Biotek, USA). ALP enzyme activity was calculated using equation (2):

$$\% \text{ Increase} = \left[ \frac{(X-R)}{R} \right] \times 100 \quad (2)$$

Where, X = Absorbance of cells corresponding to the positive control and test groups

R = Absorbance of cells corresponding to the baseline group (untreated cells)

## Results and Discussion

### Assessment of Cell Viability by MTT Assay

The effect of the untreated and Biofield Energy Treated novel formulation on the proliferation of bone cells is shown in Figure 1. The positive control,  $\beta$ -estradiol showed the cell viability by 95.51%, 89.27%, and 72.83% at the concentration of 10, 25, and 50  $\mu$ g/mL, respectively. The untreated test formulation revealed 86.06%, 97.90%, 93.53%, 93.87%, 109.99%, 70.95%, 79.93%, 83.46%, 81.11%, and 112.67% cell viability at the concentrations of 0.001, 0.002, 0.003, 0.006, 0.013, 0.025, 0.05, 0.1, 1, and 10  $\mu$ g/mL, respectively. Besides, the Biofield Energy Treated test formulation showed 112.34%, 74.69%, 70.36%, 97.57%, 72.71%, 106.63%, 117.63%, 116.46%, 111.08%, and 105.54% cell viability. Overall, data suggests that the Consciousness Energy Healing Treatment significantly increased the cell viability by 30.54%, 50.29%, 47.17%, 39.54%, and 36.95% at the concentration of 0.001, 0.025, 0.05, 0.1, and 1  $\mu$ g/mL, respectively in the Biofield Treated test formulation group as compared to the untreated test formulation group. Numerous works of literature indicated that an alternative Energy Treatment had improved

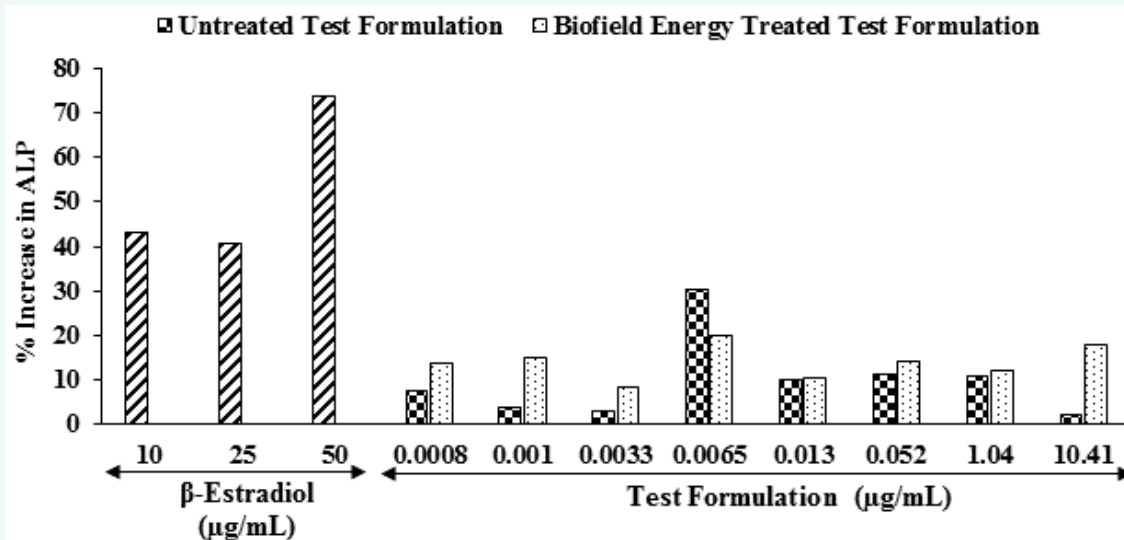
the cell viability to some extent in in-vitro cells [34-36]. Based on that, it is assumed that in this experiment the improvement of cell viability could be due to the impact of The Trivedi Effect<sup>®</sup> - Biofield Energy Healing Treatment. Overall, the test formulation showed more than 70% cell viability at the concentration ranges from 0.001 to 10  $\mu$ g/mL and indicated as safe and non-cytotoxic and selected for further analysis (Figure 1).

### Assessment of Alkaline Phosphatase (ALP) Activity

Alkaline phosphatase (ALP) is a vital component in hard tissue formation and it is highly expressed in mineralized cells. Increased level of ALP provides a fundamental mechanism of hard tissue formation and simultaneously indicated a therapeutic opportunity for the treatment of various bone diseases [37,38]. Effect of the untreated and Biofield Energy Treated test formulation on the level of ALP in human bone osteosarcoma cell (MG-63) is presented in Figure 2. The ALP level was significantly increased by 42.94%, 40.68%, and 73.82% in the positive control ( $\beta$ -estradiol) group (G2) at the concentration of 10, 25, and 50  $\mu$ g/mL, respectively as compared to the untreated cells group (G1). The level of ALP was significantly increased by 80.16%, 292.91%, 200.36%, 22.24%, and 690.35% in the Biofield Energy Treated test formulation group (G4) at 0.0008, 0.001, 0.0033, 0.052, and 10.41  $\mu$ g/mL, respectively as compared to the untreated test formulation group (G3). ALP plays a vital role in bone health. Mechanistically, the enzyme can split-up organic phosphate into inorganic phosphate (Pi). This Pi combined with the calcium ion (soluble) and formed calcium phosphate. After availability of sufficient concentration of calcium phosphate, it chemically precipitated into the osteoid [39,40]. In this experiment, the Biofield Energy Treated test formulation significantly increased the level of ALP expression which might be due to Consciousness Energy Healing Treatment (The Trivedi Effect<sup>®</sup>) that stimulate bone formation and suppress differentiation of osteoclasts so as to reduce bone resorption (Figure 2).

## Conclusions

The cell viability was evaluated using MTT assay in human bone osteosarcoma cells (MG-63). The data found that the novel proprietary test formulation showed more than 70% viable cells, which signifies a safe and nontoxic nature. Further, the level of ALP was significantly increased by 80.16%, 292.91%, 200.36%, 22.24%, and 690.35% in the Biofield Energy Treated test formulation at 0.0008, 0.001, 0.0033, 0.052, and 10.41  $\mu$ g/mL, respectively as compared to the untreated test formulation. As a result of that phosphorus absorption and deposition in bone cells can be increased which can help to make stronger bone. The data demonstrated that the Biofield Treatment has the strong potential for the treatment of patients with arthritis, osteoporosis and other bone disorders. As a result of that phosphorus absorption and deposition in bone cells can be increased which can help to make stronger bone. The data demonstrated that the Biofield Treatment has the strong potential for the treatment of patients with arthritis, osteoporosis and other bone disorders. Thus, Consciousness Energy Healing can be useful as a bone cells growth promoter for different bone-related disorders. Overall, the Biofield Energy Treated test formulation significantly



**Figure 2** Effect of the novel test formulation on human bone osteosarcoma cells (MG-63) for the assessment of alkaline phosphatase (ALP) measured at 405 nm. G1: Untreated cells; G2: Positive control ( $\beta$ -Estradiol); G3: Untreated test formulation. G4: Biofield Energy Treated test formulation.

enhanced the bone mineralization and differentiation compared to the untreated test formulation group in MG-63 cells. In conclusion, Consciousness Energy Healing Treatment can be very effective on bone health and it can be used as a complementary and alternative treatment for the prevention of various types of skeletal abnormality viz. limb abnormalities, osteoporosis, polydactyly, microcephaly, club foot, cleft hand and cleft foot, congenital hip dislocation, skull abnormalities, syndactyly, encephalocele, anencephaly, etc.

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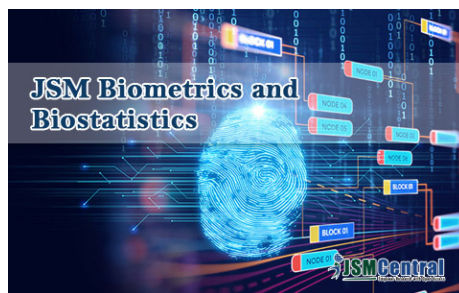
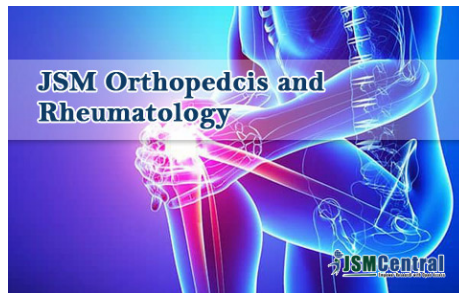
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